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CONTACT ALLERGY IN SWEDISH ADOLESCENTS: RESULTS FROM THE BAMSE COHORT STUDY

Maria Lagrelius

Karolinska Institutet

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Contact allergy in Swedish adolescents: results from the BAMSE cohort study

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By

Maria Lagrelius

Principal Supervisor:
Professor Emeritus Carola Lidén
Karolinska Institutet
Institute of Environmental Medicine

Co-supervisors:
Associate Professor Anna Bergström
Karolinska Institutet
Institute of Environmental Medicine

Professor Carl-Fredrik Wahlgren
Karolinska Institutet
Department of Medicine, Solna

Associate Professor Inger Kull
Karolinska Institutet
Department of Clinical Science and Education, Södersjukhuset

Opponent:
Professor Emeritus Chris Anderson
Linköping University
Department of Clinical and Experimental Medicine

Examination Board:
Senior Professor Lennart Emtestam
Karolinska Institutet
Department of Medicine, Huddinge

Associate Professor Lina Hagvall
University of Gothenburg
Department of Dermatology and Venereology

Professor Eva Skillgate
Sophiahemmet University
Associate Professor
Karolinska Institutet
Institute of Environmental Medicine

To my family

ABSTRACT

Background: Contact allergy affects about 20-25% of adults in the general population, but it is not completely clear how common it is among children and adolescents. Contact allergy is caused by skin contact to sensitizing substances. Knowledge about the relation between skin exposures, related skin symptoms and contact allergy among children and adolescents is limited. Atopic dermatitis (AD) and filaggrin gene (*FLG*) mutations have been suggested as risk factors for contact allergy, though this needs to be further explored.

Aims: To determine the prevalence of contact allergy at age 16 in a population-based cohort. To determine the prevalence of self-reported skin exposures and skin symptoms at age 16, and assess their association with contact allergy. To assess the association between AD at preschool age and contact allergy at age 16, and the association between *FLG* mutations and contact allergy, self-reported hand eczema and dry skin at 16 years.

Methods: We used data from a Swedish population-based birth cohort (BAMSE), followed from birth to age 16. Adolescents answered questions about skin exposures and skin symptoms at age 16 years. Their parents completed questionnaires at baseline, when the child was 2 months old and then regarding AD at 1, 2, 4, 8, 12 and 16 years. Information about contact allergy was collected by patch test (n=2,285), and *FLG* mutation status was determined from blood samples at age 16.

Results: Contact allergy prevalence was 15.3% among adolescents and higher among girls than boys (17.0% versus 13.4%, p=0.018). Nickel was the most frequent cause of contact allergy (7.5%), followed by fragrance mix I (FM I) (2.1%). Nickel allergy was more common among girls (9.8% versus 4.9%, p<0.001). Many adolescents reported piercing (55.4%) and hair dyeing (50.1%), and girls frequently reported related skin symptoms. Reports of piercing and itchy rash from metal items were associated with increased OR for nickel allergy (adjusted OR 1.77, 95% CI 1.04-3.03 and adjusted OR 2.25, 95% CI 1.57-3.23, respectively). Reported itchy rash from use of makeup or personal hygiene products was associated with increased OR for fragrance allergy (adjusted OR 2.11, 95% CI 1.02-4.35). AD at preschool age was associated with fragrance allergy (adjusted OR 3.10, 95%CI 1.66-5.80), but not with nickel allergy. This association was present among individuals with AD at preschool age with IgE sensitization, but not among individuals with AD at preschool age without. *FLG* mutations appeared unassociated with contact allergy and hand eczema, but were associated with dry skin at age 16 (adjusted OR 1.50, 95% CI 1.02-2.15).

Conclusions: Contact allergy prevalence is high among adolescents in Sweden. Nickel allergy is the most common contact allergy, affecting more girls than boys. Piercing and hair dyeing were reported by the majority at 16 years. More girls than boys reported skin symptoms related to skin exposures. AD at preschool age may be associated with contact allergy to fragrance at 16 years. No association was observed between AD at preschool age and nickel allergy. *FLG* mutations were associated with dry skin, but not with contact allergy or hand eczema at age 16 years.

LIST OF SCIENTIFIC PAPERS

I. **Lagrelius M,** Wahlgren CF, Matura M, Kull I, Lidén C. High prevalence of contact allergy in adolescence: results from the population-based BAMSE-birth cohort. Contact Dermatitis 2016; 74: 44-51.

II. **Lagrelius M**, Wahlgren CF, Matura M, Bergström A, Kull I, Lidén C. A population-based study of self-reported skin exposures and symptoms in relation to contact allergy in adolescents. Contact Dermatitis 2017; 77: 242-249.

III. **Lagrelius M**, Wahlgren CF, Matura M, Bergström A, Kull I, Lidén C. Atopic dermatitis at preschool age and contact allergy in adolescence: a population-based cohort study. Br J Dermatol 2018 Nov 22. Doi: 10.1111/bjd.17449 (Epub ahead of print).

IV. **Lagrelius M**, Wahlgren CF, Bradley M, Melén, Kull I, Bergström A, Lidén C. Filaggrin gene mutations in relation to contact allergy and hand eczema in adolescence. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

AD	Atopic dermatitis
CI	Confidence interval
FLG	Filaggrin gene
FM I	Fragrance mix I
FM II	Fragrance mix II
ICDRG	International Contact Dermatitis Research Group
IgE	Immunoglobulin E
MCI/MI	Methylchloroisothiazolinone/methylisothiazolinone
OR	Odds ratio
PPD	*p*-Phenylenediamine
PTBP-FR	*p*-tert-Butylphenol formaldehyde resin
TEWL	Transepidermal water loss

1 BACKGROUND

Contact allergy can develop at any time during life and it is life-long. When individuals are sensitized to a contact allergen, problems with allergic contact dermatitis and hand eczema can arise in the future and affect work ability for many years. Relatively little is known about contact allergy prevalence among children and adolescents, and which contact allergens they are sensitized to. Most previous studies on contact allergy among children and adolescents were performed among patients in a clinical setting; thus more information is needed about the prevalence and characteristics of contact allergy among the general population. Contact allergy develops after exposure of the skin to sensitizing substances, and the knowledge about skin exposures, skin symptoms and their relation to contact allergy among adolescents is limited. Moreover, the role of atopic dermatitis (AD) and filaggrin gene (*FLG*) mutations as risk factors for contact allergy is still not clear. New knowledge about contact allergy in adolescence might highlight current possibilities to prevent sensitization and harmful skin exposures and thus allergic contact dermatitis and hand eczema among children and adolescents and by extension also adults.

1.1 CONTACT ALLERGY

In the adult population the prevalence of contact allergy is estimated to be 20-25 %, and nickel is by far the most common cause of contact allergy (1). The metals cobalt and chromium are also common causes of contact allergy, as are perfume substances, preservatives, and chemicals in rubber, plastic and hair dye (1, 2). It is well known that contact allergy is more common among women in adult populations (3, 4). Contact allergy can result in allergic contact dermatitis if the individual with contact allergy is exposed to the triggering substance (5).

There are about 4000 known contact allergens and standard procedure for diagnosing contact allergy in patients at a dermatology clinic is by an epicutaneous test (also called skin patch test). With a skin patch test, plasters with standardized concentrations of substances, are applied on the upper back. The test needs to be attached to the skin for 2 days and then read two times, after 2, 3 or 4 and 6-7 days. According to the International Contact Dermatitis Research Group (ICDRG)-criteria, patch test reactions are assessed as +, ++, +++, ? or – (5). The patch test reactions are assessed by a dermatologist on the basis of morphology and a positive reaction is one that fulfills the criteria for at least + (5).

Baseline series for patch testing are generally used routinely in dermatology clinics. The European baseline series includes 30 patch test substances and mixes that covers around 50 common contact allergens (6). The Swedish and other local baseline series may deviate from the European baseline series, adding, omitting or replacing individual substances and adjusting concentrations to suit local needs. The substances that are suggested for inclusion in baseline series often elicit reactions from 0.5-1% of consecutively patch tested dermatitis patients at the dermatology clinic. The substance is then included if it is common in the environment and/or if it has high clinical relevance. When a patient has a positive patch test,

the relevance of the patch test reaction must be interpreted to verify the contact allergy diagnosis. A patch test reaction can have a current, past or unknown relevance (5). After the diagnosis, the patient is provided information about the specific contact allergen and common sources of exposure and thus how to avoid skin exposure and prevent development of allergic contact dermatitis. The patch test is also a necessary step in the diagnosis of allergic contact dermatitis. Early diagnosis is beneficial, and avoidance of harmful skin exposure to the skin sensitizing substance can reduce the risk of allergic contact dermatitis and hand eczema.

Patch test studies are often performed in a clinical setting among patients in dermatological specialist care, but studies in the general population are sometimes performed with simplified patch test procedures to ensure high participation and make the studies possible to perform in a population-based setting (7).

1.1.1 Immunology

Contact allergy is a delayed hypersensitivity type 4, T cell mediated reaction, to a chemical substance (8). Chemical substances that can cause contact allergy are called haptens (Figure 1). These are small molecules generally with a molecular weight of less than 500 Dalton, small enough to cross stratum corneum, the outermost layer of the skin, and penetrate into deeper layers of the epidermis (9). Haptens become immunogenic by binding to epidermal proteins (haptenization) and thus become recognizable by the immune system (10). When a skin sensitization to a chemical substance has occurred, an allergic contact dermatitis will appear if the individual is re-exposed somewhere on the skin. Allergic contact dermatitis appears if the dose on the skin is high enough and the individual's threshold of elicitation needs to be exceeded (5).

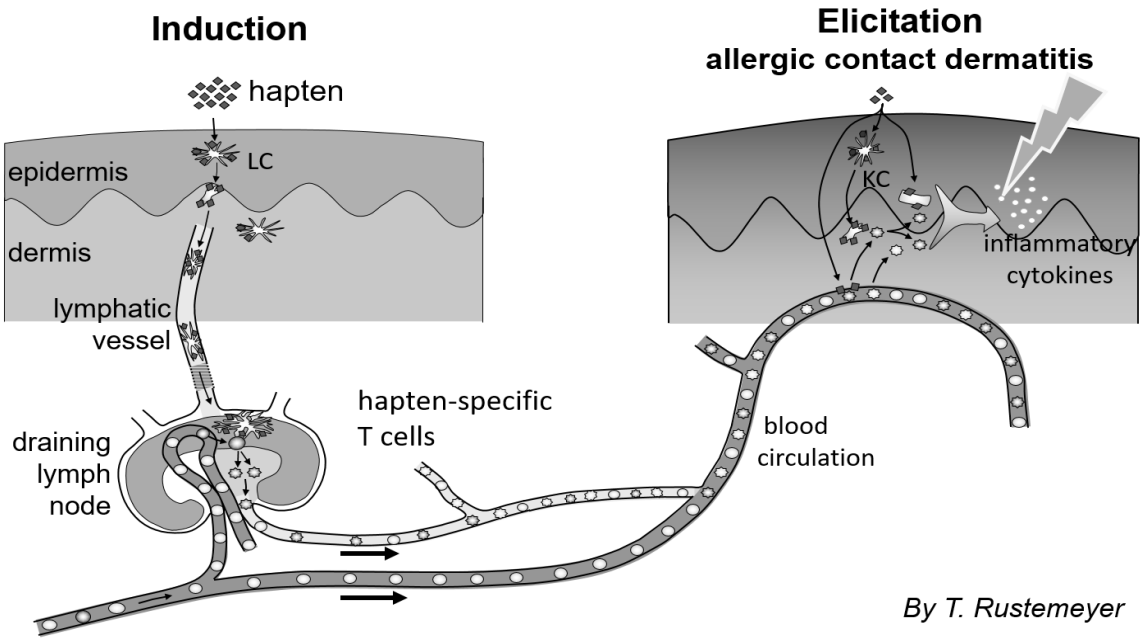

Figure 1. Illustration showing the two phases of skin sensitization: induction of contact allergy, and after re-exposure, elicitation resulting in allergic contact dermatitis. LC Langerhans cell, KC Keratocytes (Illustration adapted from T. Rustemeyer, used with permission).

1.1.2 Etiology and risk factors

A skin sensitizing chemical can also be called a contact allergen. Repeated or prolonged skin exposure to a contact allergen can cause contact allergy or elicit allergic contact dermatitis in individuals already sensitized to that specific contact allergen (5). Within the contact allergic reaction, two distinct phases are defined: the induction phase when the sensitization occurs and the elicitation phase when the allergic contact dermatitis develops (Figure 1). It is not clear whether individual co-factors such as genetic factors or co-morbidities confer a greater risk for development of contact allergy.

Women are known to have a greater risk for development of contact allergy to nickel. This is thought to be due to fashion related adornment behaviors that result in greater skin exposure to nickel, and not genetic differences or hormonal factors related to sex (11). Women are also known to have disproportionately frequent occupational skin exposure to water and are more affected by hand eczema that results in a defect skin barrier which is known to exacerbate the risk of contact allergy (12, 13).

1.1.3 Clinical features and treatment

Contact allergy is defined by a positive patch test reaction, and thus a contact allergy diagnosis is established. However, allergic contact dermatitis is the disease that causes the clinical problem. Allergic contact dermatitis is a localized rash that appears on the surface of the skin that has been exposed to a contact allergen in an already sensitized individual. Redness, blisters, edema, papules, scales and itch can occur. Any part of the body that is exposed to the contact allergen can be affected by allergic contact dermatitis (14, 15).

Contact allergy is life-long, and to prevent development of allergic contact dermatitis, sensitized individuals should avoid skin contact with responsible contact allergen(s). Avoidance of skin exposure can be difficult; many individuals with contact allergy are unaware of their allergy because they have not been patch tested and moreover because of insufficient ingredient labelling on products (16). Allergic contact dermatitis is usually treated with topical glucocorticoids. Moisturizers are often used to treat dry skin and this treatment strengthens the skin barrier.

1.2 CONTACT ALLERGY IN CHILDREN AND ADOLESCENTS

Most of our knowledge about contact allergy among children and adolescents is based on studies among patients within dermatological specialist care (17-21). Only a few studies have been performed in the general population, and many of them are small because patch testing requires a lot of resources and time (22-29). Studies in the general population enable estimation of contact allergy frequency among that population as a whole, but more knowledge is needed to ascertain the magnitude of contact allergy among adolescents. Determining the prevalence of contact allergy among adolescents and which contact allergens they are sensitized to may enable us to take action already in childhood and adolescence and prevent skin exposure and development of contact allergy.

Prior to the studies presented in this thesis, a large population-based study was performed among adolescents in Denmark, 1,146 of whom were patch tested. That study reported that 15.2% of school children aged 12-16 years had contact allergy and 8.6% were sensitized to nickel (22). A Swedish study patch tested 4,376 teenagers for nickel, and showed 9.9% prevalence of nickel allergy (30). A recent meta-analysis performed on studies among the general population showed an overall prevalence of 16.5% (95% CI 13.6-19.7) among children and adolescents < 18 years of age (7). Children and adolescents are exposed to skin sensitizing substances through various consumer products and topical treatments, and their contact allergy pattern often reflects their skin exposure to skin sensitizing chemicals in their environment (18, 31). It is thus important to improve knowledge about skin exposures and skin symptoms and their relation to contact allergy among children and adolescents.

The clinical presentation involves a localized rash in both adults and children, but since AD is prevalent among the pediatric patients, allergic contact dermatitis might be falsely interpreted as AD (32). Hyposensitization treatment or desensitization can be attempted against IgE mediated allergies, but not against contact allergy: avoidance of skin contact with the contact allergen is the only option. The consequences of contact allergy may thus be greater for children and adolescents than for adults since contact allergy, once established, requires life-long avoidance of the allergen and this avoidance of skin exposure might affect everyday life negatively (33).

1.2.1 Nickel allergy

Nickel allergy is the most common contact allergy among children and adolescents. The prevalence of nickel allergy in the general population in Europe is around 8-18% (34). An association between nickel allergy and hand eczema has been shown among both adults and adolescents (35, 36). The diverging results reported in studies might reflect decreasing skin exposure, due to the EU restriction of nickel release and also previous national legislation (1, 35, 37). The nickel restriction entered into full force in 2001 in the EU and aimed to reduce nickel allergy in the general population in Europe. Items intended for prolonged contact with the skin must not release more than 0.5µg/ cm^2/week when immersed in artificial sweat. In April 2014, the European Chemicals Agency (ECHA) defined prolonged contact as "potentially more than 10 minutes on three or more occasions within two weeks, or 30 minutes on one or more occasions within two weeks" (38). The levels of nickel exposure vary in different countries and the prevalence of nickel allergy is lower in countries like Sweden and Denmark than in for example Italy and Poland due to differences in compliance with the EU restriction on nickel and national legislation prior to the EU restriction (3, 39-43).

It is well known that there is a female predominance of nickel allergy among adults (3, 4). Studies among both patients with dermatitis and the general population show diverging results on whether this difference is evident already in childhood (23, 24, 44). For many years it has been stated that both piercing itself and wearing jewellery in pierced holes increase the risk for contact allergy to nickel (45-47). The risk is probably less prominent now in countries with good compliance with the EU restriction (39). Cobalt sensitization has previously been

described as often being concomitant with nickel sensitization, but solitary contact allergy to cobalt can also occur frequently (4, 48, 49).

1.2.2 Fragrance allergy

Perfumed cosmetic products can contain more than 2500 substances or natural extracts that are defined as fragrances (50). Fragrance allergy is currently tested for with: fragrance mix I (FM 1), fragrance mix II (FM II) and *Myroxylon pereirae* that is a natural mixture, in the Swedish baseline series. The European baseline series additionally tests for a single synthetic fragrance chemical, namely hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC, Lyral ®) which is also included in FM II. This procedure tests for markers of fragrance allergy, but does not cover all contact allergies to fragrance substances (51).

FM I contains a mix of fragrance substances commonly present in perfumed products (cinnamyl alcohol, cinnamal, hydroxycitronellal, amyl cinnamal, geraniol, eugenol, isoegenol and oakmoss absolute). Contact allergy to fragrance substances in FM I is one of the most frequent contact allergies in children and adolescents as well as in adults (7, 52-55). The prevalence of contact allergy to FM I is estimated to be around 1-3% in the general population (2, 50). Personal hygiene products found in regular stores, such as soaps, shower gels, moisturizers and wipes, often contain sensitizing fragrance substances; this is true even of products that are produced intended for children (56-58). A recent study among dermatitis patients suggests that fragrance allergy is increasing among both men and women (59). It has also been reported that the prevalence of contact allergy to fragrances is higher among pediatric patients with AD than among those without AD (60).

1.2.3 *p*-Phenylenediamine allergy

Contact allergy to *p*-phenylenediamine (PPD) appears to be increasing in the general population and this has mainly been related to hair dyeing, but also black henna tattoos and working as a hair dresser (61-63). The prevalence of contact allergy to PPD is estimated to 1% among the adult population in Europe (61). Temporary black henna tattoos can cause skin sensitization to PPD and these tattoos are popular among children and adolescents. Sensitized individuals can suffer a severe allergic contact dermatitis reaction after dyeing their hair with a hair dye containing PPD (64). PPD sensitization may also result in cross-sensitization with other compounds with chemical similarities for example chemically related hair dyes and textile dyes (62). Hair dyes can contain PPD or chemically related compounds, but they generally also contain other chemicals that are not covered by standard patch test substances (65). PPD is currently the only hair dye chemical that is included in the Swedish baseline series.

1.3 CO-FACTORS FOR CONTACT ALLERGY

1.3.1 Atopic dermatitis

AD is an inflammatory skin disease that is characterized by an itchy, red, dry skin and scratching. The itchiness mainly affects skin in the body folds. The dermatitis has a relapsing

course during childhood and sometimes continues into adulthood. It has been estimated that AD affects 2-10% of adults and 15-30% of children (66). AD usually presents already in early childhood (67). It has previously been reported that AD in early childhood is associated with IgE sensitization (68). However, the role of AD as a potential risk factor for development of contact allergy is not clear. It has been suggested that the deterioration of the skin barrier in individuals with AD could heighten the risk of penetration also of contact allergens and thus the risk of skin sensitization. In addition, children with AD may be frequently exposed to contact allergens through topical treatments like moisturizers, glucocorticoids and ointments (69-72). Studies show diverging results, and association between AD and contact allergy has not been consistently confirmed (73, 74).

1.3.2 Hand eczema

Hand eczema is defined as dermatitis localized on the hands. The one-year prevalence of hand eczema is approximately 5% among adolescents and 10% among adults in the population (75, 76). Previous or current AD, contact allergy and wet work with skin exposure to irritants, are the most important risk factors for developing hand eczema. Hand eczema is known to be the most frequent skin disease that is work-related and results in lower health-related quality of life, long treatment periods, negative socio-economic consequences for both the individual and society, and it may be difficult for individuals affected by hand eczema to continue their current employment (77-79). Contact allergy has been shown to be more common in children with AD presenting with dermatitis on hands and/or feet (80). An association has also been reported between hand eczema and contact allergy among Danish adolescents (22). The role of *FLG* mutation in relation to hand eczema in adolescence has not been fully investigated.

1.3.3 Dry skin

Dry skin can be measured at a clinical examination or by self-reports of symptoms in a questionnaire (81). A dysfunctional skin barrier function can be assessed by measuring transepidermal water loss (TEWL) (82). Heightened TEWL is associated with dry skin among adult patients with AD (83). Dry skin is also an important diagnostic criterion for AD, both for the dermatologist in the clinical setting and for researchers in epidemiological studies (84). Dry skin can result in an impaired skin barrier and thus dry skin may be a risk factor for contact allergy, if it facilitates penetration of contact allergens. Moreover, moisturizers are routinely used to treat dry skin and are often applied repeatedly and during long time. Moisturizers are known to contain contact allergens and can thus also increase risk of developing contact allergy (85).

1.3.4 Filaggrin gene mutations

FLG mutations have been investigated when trying to determine genetic factors associated to development of contact allergy. Filaggrin (filamin aggregating protein) is an epidermal protein that is important for skin structure and function. Profilaggrin is encoded by the *FLG*.

Profilaggrin is later cleaved into filaggrin peptides that participate in the keratinization of the skin (86).

It is known that *FLG* mutation carriers can develop dry skin, ichtyosis vulgaris, and that they are more likely to develop AD (86, 87). Loss-of-function *FLG* mutations have been identified as a cause of ichtyosis vulgaris. This disease is characterized by extremely dry and scaly skin (86). Over 40 different *FLG* mutations have been detected. *FLG* mutations are population-specific and differences among the mutations have been described in different regions of the world (88). Mutations in the *FLG*, R501X, R2447X and 2282del4, are common in northern Europe (89). It has been estimated that R501X and 2282del4 are present in around 9% of the European population (87).

The role of *FLG* mutations in development of contact allergy and hand eczema is not clear. Some studies suggest that mutation carriers also have an increased risk of developing nickel allergy and a lower age at onset of eczema due to nickel allergy (90-92). In contrast, other studies found association between AD and *FLG* mutations, but not between *FLG* mutations and contact allergy or hand eczema (93, 94). *FLG* mutations have previously been reported to be associated with dry skin during childhood (47, 89, 95).

1.3.5 IgE sensitization

Allergy mediated by IgE antibodies is called IgE-mediated allergy (96). The immune system reacts to an allergen by producing IgE antibodies and these can later be detected in the blood. Allergen specific IgE antibodies, for example to food or pollen, can also exist in individuals without clear clinical manifestations of allergic disease (97). IgE sensitization to inhalant allergens or food allergens is referred to as atopy, according to the World Allergy Organization (WAO) nomenclature (96). Theoretically the impaired skin barrier in individuals with AD can result in penetration of protein allergens which are common causes of IgE mediated allergy, as well as penetration of contact allergens. Moreover, it has been shown that adolescents with AD and concomitant IgE sensitization present with more severe AD (98). Presence of IgE sensitization can be a marker of a defective skin barrier function and may also indicate a risk for development of contact allergy (99, 100). The interplay between different co-factors is complex and they do coexist (100, 101).

1.4 CERTAIN SKIN EXPOSURES

Skin exposure is mandatory for developing contact allergy and allergic contact dermatitis on the hands, face, or other exposed body part. Adornment customs and fashion trends among adolescents can lead to harmful skin exposure to sensitizing substances, due to for example piercing, hair dyeing and tattooing (102). Skin exposure to contact allergens can also vary in magnitude in different countries and cultures. Skin exposures may for example be related to cultural adornment behaviors, herbal medicine, laws and restrictions in a country, treatment policies for topical medicaments and the local assortment of consumer products (62, 65, 103). Skin exposures to fragrances are substantial since many consumer products, such as make up, and products for personal hygiene and cleaning, contain fragrance substances. Fragrance

substances are also found in products intended for children and products marked as hypoallergenic (56, 104). Common sources of skin exposure to various substance groups are exemplified in Table 1.

Table 1. Examples of common sources of skin exposure to the contact allergens in different substance groups.

Substance group	Sources of exposure
Metals	Jewellery, belt buckles, coins, leather
Adhesive chemicals	Plasters, cosmetics, shoes, glue
Fragrances	Deodorant, shampoo, soap, detergents
Topical drugs	Topical treatments, moisturizers
Hair dye substances	Hair dye, black henna tattoo, eyebrow dye
Preservatives	Cosmetics, cleansing wipes, soap, paint
Rubber chemicals	Gloves, shoes, boots

1.4.1 Piercing

Piercing is when a hole is made through the skin of the earlobe or any other part of the body to wear jewellery. Adornment by piercing and wearing jewellery for pierced holes is one frequently occurring lifestyle factor that can entail skin exposure to contact allergens like metals and pose a risk of nickel allergy (1, 47, 105). In previous Swedish surveys, 86-90% of teenage girls and 13-21% of teenage boys report piercing (46, 47, 105). A recent survey of body piercings in France showed that piercing of ear, navel, tongue and nose were most common (103). The risk of developing contact allergy by wearing jewellery for pierced holes is affected by the material in the jewellery and the risk of nickel allergy has been shown to be lower among teenagers in Denmark after the Danish restriction on nickel, preceding the EU restriction on nickel (106).

1.4.2 Hair dyeing

Hair dyeing is common in the general population both among adolescents and adults. Both skin reactions after hair dye and the use of hair dye is increasing. This increase is reported in the general population in Sweden and Denmark and increased prevalence of PPD reactions has been described in England (105, 107, 108). Sixty-six percent of 16-year-old girls report hair dyeing and 17% of 16-year-old boys (105); 3.5% of 16-year-old girls report skin reaction after hair dye use (105). Hair dye (oxidative and non-oxidative) is known to contain potentially skin sensitizing substances for example PPD, toluene-2,5- diamine sulfate and

resorcinol. Hair dye is often used repeatedly and therefore poses a risk for developing contact allergy (109). Contact allergy to PPD is often related to hair dye use (62).

1.4.3 Tattooing

Tattooing represents a known risk for developing contact allergy (102, 110). Permanent tattoos are not so frequent among children and adolescents; only a few 12-year-olds and less than 1% of 16-year-olds reported having a permanent tattoo in an environmental health survey in Sweden (105). Among young adults and adults in the general population of Sweden about 17% reported a permanent tattoo (111). Tattoo inks are known to contain contact allergens like metals and hazardous chemicals like azo dyes, aromatic amines and preservatives (112). Tattooing a child is not illegal in Sweden, but proper consent from the guardian is mandatory before performing a tattoo on individuals younger than 18 years (113).

1.4.4 Black henna tattooing

Black henna tattoos are tattoos painted on the skin and these tattoos can contain skin sensitizing substances such as PPD and other hair dye chemicals. Black henna tattoos are popular among children and adolescents; 26% of 16-year-old girls and 16% of 16 year-old-boys in the general population of Sweden report that they have had a temporary black henna tattoo at some time (105). In the general population, individuals who have had a black henna tattoo show a higher prevalence of contact allergy to PPD than individuals who have never had a black henna tattoo (61).

2 AIMS

The overall aim of this thesis was to study contact allergy according to patch test in Swedish adolescents within a population-based birth cohort. Additional aims were to study contact allergy in relation to skin symptoms, skin exposures, atopic dermatitis, hand eczema and filaggrin gene mutations.

In particular:

To determine the prevalence of contact allergy at age 16 years, to identify the most common contact allergens, and to describe differences in prevalence between girls and boys.

To determine the prevalence of certain self-reported skin exposures and skin symptoms at age 16 years, and to assess their association with contact allergy.

To assess the association between atopic dermatitis at preschool age and contact allergy at age 16 years.

To assess the association between filaggrin gene mutations and contact allergy, self-reported hand eczema and self-reported dry skin at 16 years.

3 MATERIALS AND METHODS

3.1 BAMSE BIRTH COHORT

All the studies included in this thesis (I-IV) are based on data from the BAMSE (Swedish acronym for Children (Barn), Allergy, Milieu, Stockholm, Epidemiology) study. The BAMSE study is a longitudinal population-based birth cohort that was initiated to study environmental risk factors for developing allergic diseases in children (114).

3.1.1 Recruitment

The study participants were recruited consecutively as newborns from child health care centers in four areas of Stockholm: northwestern districts near the city center (Norrmalm and Vasastan), Solna, Sundyberg and Järfälla municipalities. Geographically, the predefined areas form a circular sector of the city, and were chosen to include a varied distribution of socioeconomic parameters and housing conditions.

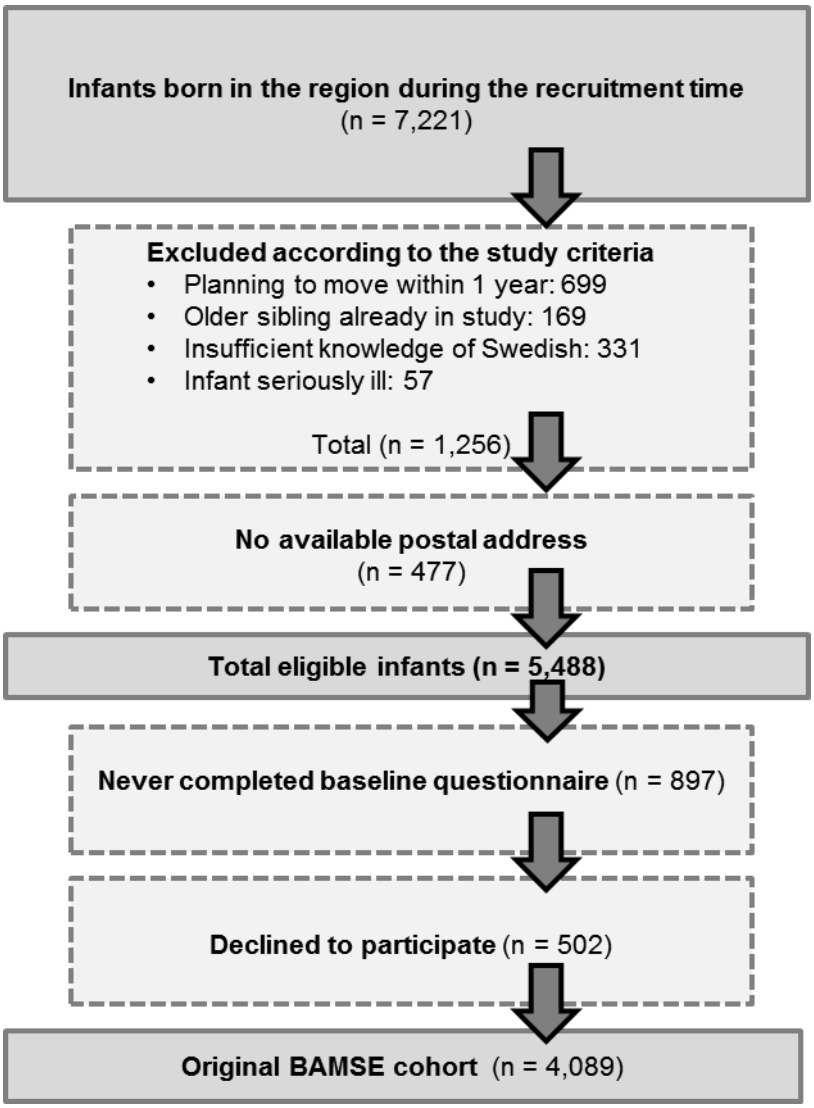

Figure 2. The recruitment of the original BAMSE cohort in the BAMSE study (114).

The recruitment started February 1994 and continued to November 1996. The parents of all children born in the recruitment region were contacted from child health care centers and through a community population register, and asked to participate in the study. During the recruitment period 7,221 children were born in the study area, 5,488 were eligible according to the criteria for inclusion; 4,089 children were included to the final cohort, and these children represent 75% of the eligible children (Figure 2). Their parents answered the baseline questionnaire when their children were a median of two months old.

To investigate the representativeness of the final original BAMSE cohort, non-responders to the baseline questionnaire and the actively excluded families were sent a short questionnaire to evaluate distribution of selected exposures and parental allergic disease in 1996. Sixty-seven percent responded and the results of that study showed that parental smoking, when the child was 2-4 months old was more common among non-responders and actively excluded families than among the families included in the final original cohort. However, parental allergy rates and presence of pets in the household were comparable with the final original BAMSE cohort (114).

3.1.2 Baseline and follow-up

The baseline questionnaire (Q0) covered questions concerning lifestyle of the parents, residential factors, parental allergic disease, socioeconomic parameters and environmental exposures. In a longitudinal design the follow-ups were performed at approximately 1, 2, 4, 8, 12 and 16 years (Q1, Q2, Q4 etc). These follow-ups had 96%, 94%, 91%, 84%, 82% and 78%, in response rate, respectively. From the 12-year follow-up the adolescents were able to answer a questionnaire as well as the parents. From the 12-year follow-up the questionnaire was web-based. In all these follow-ups the parents (and later the adolescents) were given opportunities to answer questions regarding general health, symptoms of AD, asthma and rhinitis, use of medications and health-related quality of life as well as lifestyle factors and environmental exposures. Children were also invited to participate in a clinical examination including blood sampling at 4, 8 and 16 years.

At the 16-year follow-up, the adolescents completed questions regarding skin exposures to various consumer products and known skin- sensitizers and skin symptoms related to certain skin exposures. Detailed questions addressed body and ear piercings, tattooing, hair dyeing, skin symptoms from use of consumer products including jewellery, metal items, rubber items, cosmetics and personal hygiene products. The participants' mean age when they completed the questionnaire was 16.6 years (range 15.7-19.0 years). The questionnaires were filled in before a clinical examination that included a patch test.

3.2 PATCH TEST

Prior to the clinical examination for the 16-year follow-up the adolescents were invited to participate in a skin patch test. The patch test was performed to determine presence of contact allergy, and a TRUE test®, with 3 panels, was used (1 and 2 were the regular panels and 3 was a specially prepared panel for this study). A total of 30 substances including common

contact allergens like rubber chemicals, metals, fragrance substances, preservatives, plastic chemicals, glucocorticoids and PPD were tested. The test was sent to the participant by postal mail before the clinical examination. The test was applied at home on the upper back by the participant or family member, according to written instructions specially prepared for this study. At the clinical examination after 2 days of skin contact, the test was taken off and one hour later, the result was assessed, recorded and photographed by specially trained research nurses (Figure 3). The protocols and photos were later examined by a panel of two dermatologists to determine contact allergy. The photos were assessed under coded conditions and if there was a disagreement between the panel of dermatologists the protocol and assessment of the specially trained research nurse was taken into account and given equal importance. If there was a large discrepancy, the photos were re-assessed and final assessment was made after discussion between the two dermatologists.

The results were scored as positive, negative or inconclusive owing to technical problems (e.g. camera failure, insufficient skin contact or patch test panel falling off). The criteria for a positive patch test reaction were homogenous erythema and infiltration according to ICDRG criteria and a patch test reaction was scored as negative if it did not fulfill the criteria for a positive patch test reaction (5). A similar study design has been used in previously performed population-based patch test studies in Denmark (115, 116).

Figure 3. Showing patch test reading procedure, at clinical examination, when patch test was taken off, then after one hour, read, assessed and photographed. Positive patch test reaction to nickel.

3.3 DEFINITIONS

3.3.1 Background variables (I-IV)

These background variables were based on questionnaire data filled out by the parent when the child was median age two months (Q0), one year (Q1), and eight years (Q8), and filled out by the adolescent at 16 years (Q16) (Table 2).

Table 2. Description of the background variables in study I-IV.

Variable	Definition	Study
Parental smoking	Any parent smoking at least one cigarette daily at baseline. (Q0)	I-IV
Parental allergic disease	Mother and/or father with doctor's diagnosis of asthma and asthma medication and/or doctor's diagnosis of hay fever in combination with furred pets and/or pollen allergy and/or doctor's diagnosis of AD. (Q0)	I-IV
Socioeconomic status	White-collar worker in household, socioeconomic groups according to Statistics Sweden. (Q0)	I-IV
Parental migration status	Any parent born outside Sweden, Norway, Denmark or Finland. (Q8)	I-IV
Young mother	Mother's age < 25 years at birth of child. (Q0)	I-IV
Infantile AD	Doctor's diagnosis of AD and/or typical symptoms of AD before 1 year of age. (Q1)	I-II
Exclusive breastfeeding ≥4 months	Child was breastfed for at least 4 months without exposure to solid food or formula. (Q1)	III-IV
Smoking at 16 years	Any smoking by adolescent at 16 years, occasional or daily. (Q16)	II

3.3.2 Contact allergy (I-IV)

The variables for contact allergy were based on the skin patch test performed at the clinical examination of the 16-year follow-up (Table 3).

Table 3. Categorizing variables of contact allergy in study I-IV based on skin patch test at 16 years.

Variable	Definition	Study
Any contact allergy	Contact allergy to any of the 30 tested substances in patch test at 16 years.	I-IV
Nickel allergy	Contact allergy to nickel in patch test at 16 years.	I-IV
Fragrance allergy	Contact allergy to fragrance mix I in patch test at 16 years.	I-IV
***p*-Phenylenediamine allergy**	Contact allergy to *p*-phenylenediamine in patch test at 16 years.	I-II

3.3.3 Skin symptoms (II-IV)

The variables of skin symptoms were based on questionnaire data filled out by the parent or adolescents. Details are shown in Table 4.

Table 4. Categorizing variables of skin symptoms. AD variables in study III-IV were based on parental or adolescent reporting in questionnaire. Variables of skin symptoms related to certain exposures in study II, hand eczema and dry skin in study IV were based on adolescent reporting in questionnaire at age 16 years.

Variable	Definition	Study
AD at preschool age	Dry skin and itchy rash for ≥ 2 weeks with rash in specific locations (face or arm/leg extension surfaces or arm/leg flexures or wrist/ankles or neck) in the last 12 months and/or doctor's diagnosis of AD since the last follow-up on at least one of the follow-ups at age 1, 2 and 4 years.	III
Persistent AD	AD at preschool age and AD at 1 or more follow-up after 4 years at 8, 12 and/or at 16 years.	III
Transient AD	AD at preschool age but no AD at follow-ups after 4 years at 8, 12 or 16 years.	III
School onset AD	AD at follow-ups at the age of 8, 12 and/or 16 years, but no AD at preschool age.	III
AD at 16 years	Self-reported dry skin and itchy rash for ≥ 2 weeks with rash in specific locations (face or arm/leg extension surfaces or arm/leg flexures or wrist/ankles or neck) in the last 12 months.	IV
Itchy rash from metal	Self-reported ever having itchy rash or eczema from metal items.	II
Itchy rash from specified metal items	Self-reported ever having itchy rash or eczema from specified metal items: jewellery, jewellery for pierced body parts or metal in clothes.	II
Itchy rash from makeup or personal hygiene products	Self-reported ever having itchy rash or eczema from makeup or personal hygiene products.	II
Itchy rash from specified cosmetic products	Self-reported ever having itchy rash or eczema from specified cosmetic products: shampoo or conditioner, soap or shower gel, makeup or perfume, deodorant or other product.	II

Symptoms from hair dying	Self-reported ever having symptoms from hair dyeing.	II
Hand eczema at 16	Self-reported hand eczema in the last 12 months[a].	IV
Hand eczema ever	Self-reported ever having hand eczema[b].	IV
Dry skin at 16	Having problems with dry skin at 16 years.	IV

[a] Affirmative answer to the question "Have you had hand eczema on any occasion during the past 12 months?" This question has been validated previously (117). [b] Affirmative answer to the question "Have you ever had hand eczema (itching eruption, vesicles or itching rash)?"

Questions concerning skin symptoms related to certain exposures are presented in detail in supporting information, Table S1, to paper II.

3.3.4 *FLG* mutations (IV)

From blood samples collected at 16 years, DNA was extracted, and genotyping was performed for *FLG* mutations (R501X, R2447X, 2282del4) common in northern Europe (84). *FLG* mutations were defined as a mutation in any of the positions R501X, R2447X or 2282del4. For all three mutations, TaqMan SNP genotyping assays (Applied Biosystems, CA, U.S.A) were used. Triplicates of all samples were analyzed.

3.3.5 Skin exposures (II)

Piercing was based on adolescent reporting in questionnaire of ever piercing ears or any other part of the body for jewellery up to 16 years of age.

Hair dye was based on adolescent reporting in questionnaire of ever dyeing hair up to 16 years of age.

Tattoo was based on adolescent reporting in questionnaire of having any tattoo up to 16 years of age.

Questions concerning skin exposures related to certain exposures are presented in detail in supporting information paper II.

3.3.6 IgE sensitization (III)

Serum IgE antibodies were analyzed in blood collected at clinical examination at 4 years of age. Airborne allergens and common food allergens were analyzed using ImmunoCAP System (Thermo Fischer Scientific, Uppsala, Sweden) with Phadiatop® (cat, dog, horse, birch, , timothy, mugwort, *Dermatophagoides pteronyssinus* (house dust mite) and *Cladosporium* (mold)) and fx5 (cod, wheat, soy, cow's milk, egg and peanut). The results were considered positive at ≥ 0.35 kUA/L and negative at < 0.35 kUA/L. To be classified as IgE-sensitized a child had to have a positive result to at least Phadiatop® or fx5.

3.4 STUDY DESIGN AND STUDY POPULATIONS (I-IV)

The studies included in the thesis have an observational study design. Studies I and II are cross-sectional studies with exposures and outcomes measured at the same follow-up, while study III is longitudinal and the exposures were collected repeatedly over time. Study IV explored if *FLG* mutations had an association to development of contact allergy, hand eczema or dry skin in adolescence.

Study I included 2,285 adolescents who participated in the 16-year follow-up including skin patch test (88% of the participants in the clinical examination at the16-year follow-up) (Figure 4).

Study II included 3,115 adolescents who filled out the questionnaire at age 16 (76% of the original cohort). A subpopulation encompassing 2,285 adolescents had participated in the patch test.

Study III included 2,215 adolescents whose parents had completed questions on symptoms of AD at 1, 2 and 4 years and who were patch tested at the 16-year follow-up.

Study IV included 1,822 adolescents who had completed questions regarding symptoms of hand eczema at 16 years, were patch tested at the 16-year follow-up and had data on *FLG* mutation status.

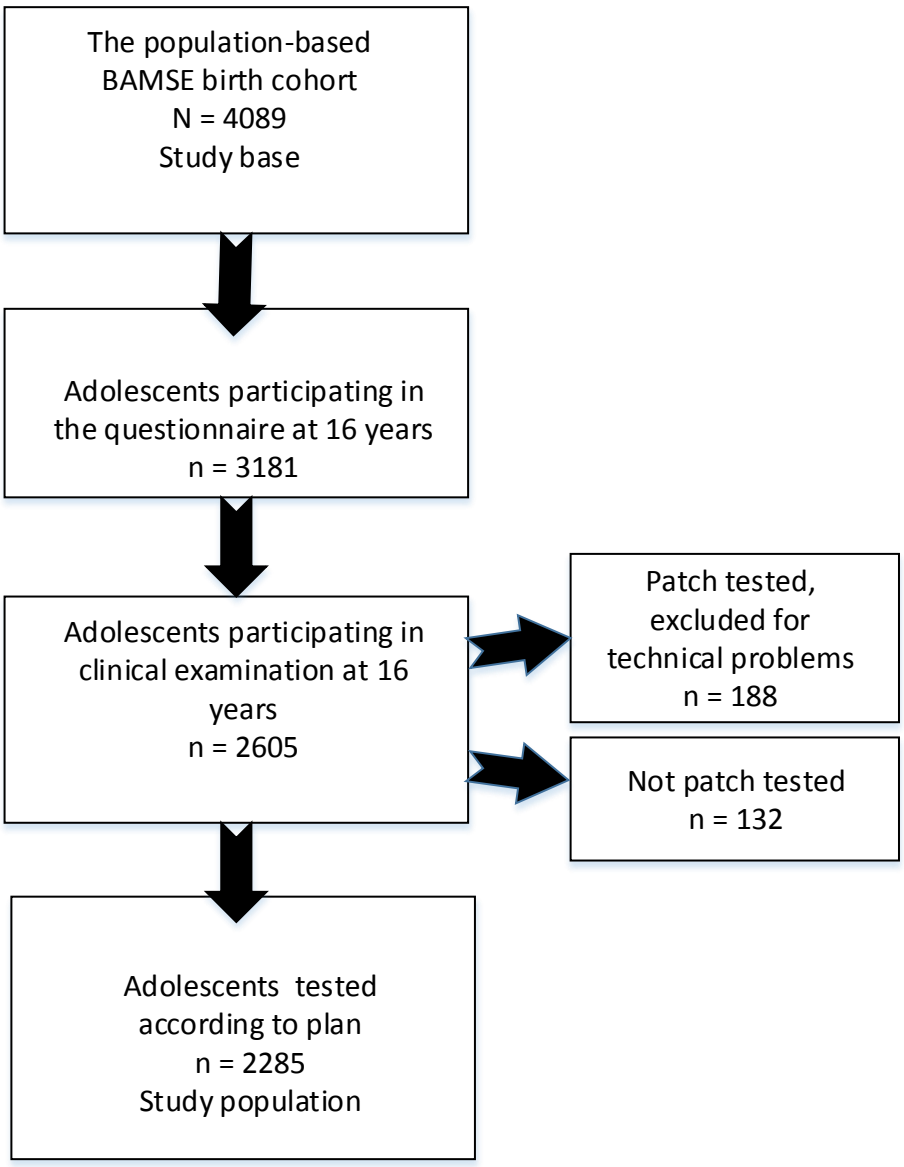

Figure 4. Flow chart showing the original BAMSE cohort and the patch tested adolescents encompassing the study population (n = 2,285) (Study I).

3.5 STATISTICAL METHODS

The statistical calculations were performed with STATA statistical software release 11.1 in study I, release 11.2 in study II and release 13 for studies III-IV.

3.5.1 Descriptive statistics

3.5.1.1 Prevalence

Prevalence is an epidemiologic term for the proportion of a population that is affected by a specific medical condition or exposure. In our studies, the prevalence proportions of contact allergy were defined as all positive patch test results divided by the total number of patch tested adolescents for that specific patch test substance (study I). The prevalence of self-reported skin exposures and symptoms were defined as all positive answers to a question divided by all adolescents answering that specific question (study II).

3.5.2 Chi2 tests (I-IV)

The chi2 test is often used to compare if there is a statistically significant difference between the expected frequency of a category and the observed frequency. We used this method for comparing dichotomous variables between two groups; chi2 tests were used in all papers included in the thesis. Differences were considered significant if p-values were less than 0.05.

3.5.3 Confidence intervals (I-IV)

Confidence intervals give an estimated range of plausible values which is likely to include an unknown variable from a given data sample; 95% confidence intervals (CI) were calculated when comparing background characteristics between percentages of total number of adolescents in the study population and the original cohort in all papers included in the thesis.

3.5.4 Logistic regression (II-IV)

A regression model can be used when analyzing associations between exposure(s) and a binary outcome (dependent variable). Logistic regression is a modeling of the odds of the outcome. It generates the odds ratio. Logistic regression was the main statistical method for association analyses for dichotomous variables in the studies of this thesis. The results are presented as odds ratios (OR) together with the 95% CI. Different confounder models were tested to evaluate potential confounders based on prior knowledge. The final adjustment model was then made for factors that changed the OR > 10% in study II and in study III for potential confounders suggested for AD. Stratified analyses for sex were made in studies II-III after interaction analysis. Stratified analyses were also made in study IV for AD at 16 and dry skin at 16. In study IV the final adjustment model was made with factors that changed the OR > 5%.

3.6 ETHICAL CONSIDERATIONS

All the follow-ups in the BAMSE study until the 8-year follow-up were approved by the Ethics committee of Karolinska Institutet, Stockholm, Sweden. The 12- and 16-year follow-ups were approved by the regional ethical review board in Stockholm. The parents of all participants and participants gave informed consent and were informed that they were able to withdraw from the study at any time. At the clinical examination both the parent and adolescent gave written informed consent to participate.

4 RESULTS

4.1 CONTACT ALLERGY PREVALENCE (I)

The study population of 2,285 patch tested adolescents in study I was comparable with the original cohort regarding background variables despite small statistically significant differences with somewhat higher participation among girls and adolescents in with parents who were white-collar workers (Table 5).

Table 5. Comparison of background variables between the study population (n=2,285) and the original cohort (n=4,089).

	Original cohort n=4,089 % (n)	**Study population n=2,285 % (n)**	**95% CI**
Sex, male	50.5 (2,065)	**47.5** (1,086)	45.5-49.6
Parental history of AD, asthma or rhinitis[a]	43.1 (1,746)	44.4 (1,015)	42.8-46.9
Parental smoking at baseline, yes[b]	21.0 (855)	20.0 (455)	18.4-21.7
Socioeconomic status[c] **white-collar worker**	82.7 (3,323)	**84.7** (1,910)	83.3-86.2
Infantile eczema[d]	15.1 (594)	15.4 (345)	13.9-16.9
Young mother[e]**, yes**	7.8 (319)	7.3 (167)	6.2-8.4

[a] Doctor's diagnosis of asthma and asthma medication and/or doctor's diagnosis of hay fever in combination with furred pets and/or pollen allergy and/or doctor's diagnosis of AD at baseline in any parent. [b] Any parent smoking at baseline. [c] Socioeconomic groups according to Statistics Sweden. [d] Doctor's diagnosis of atopic dermatitis and/or typical symptoms of atopic dermatitis before 1 year of age. [e] Mother's age <25 years at birth of the child. Statistically significant differences are shown in bold.

Of all the patch tested adolescents, 15.3% had at least a positive reaction to one of the 30 patch tested substances. The prevalence of contact allergy was higher among girls than boys (17.0% versus 13.4%, p=0.018). Nickel gave the highest frequency of positive reactions among the 30 tested substances (7.5%) followed by FM I substances (2.1%), *p*-tert-butylphenol formaldehyde resin (PTBP-FR) (1.9%), cobalt (1.2%) and PPD (1.1%). The nickel allergy prevalence was significantly higher among girls (9.8% versus 4.9%, p<0.001). The prevalence of contact allergy to *Myroxylon pereirae* was significantly higher among boys (0.7% versus 0.1%, p = 0.024). Table 6 describes in detail the prevalence of contact allergy to the contact allergens and mixes that most frequently caused contact allergy.

Table 6. Contact allergy prevalence for the 10 substances and mixes that most commonly caused contact allergy listed by frequency of positive reactions at day 2 in Swedish adolescents patch-tested at 16 years in the BAMSE cohort (n=2,285) (Adapted from paper I).

Substance	Prevalence (%)			
	All	**Girls**	**Boys**	***p***
Nickel sulfate	7.5	9.8	4.9	<0.001
Fragrance mix I	2.1	1.8	2.4	ns
PTBP-FR	1.9	1.9	1.9	ns
Cobalt chloride	1.2	1.2	1.1	ns
PPD	1.1	1.1	1.0	ns
Colophonium	0.4	0.6	0.3	ns
Lanolin alcohol	0.4	0.4	0.3	ns
Myroxylon pereirae	0.4	0.1	0.7	0.024
MCI/MI	0.3	0.3	0.3	ns
Potassium dichromate	0.3	0.4	0.3	ns
Any positive reaction	15.3	17.0	13.4	0.018

ns, not significant; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; PPD, *p*-phenylenediamine; PTBP-FR, *p*-tert-butylphenol formaldehyde resin. *p* for differences between girls and boys.

Solitary contact sensitization to cobalt was more common than co-sensitization with nickel. Of the cobalt sensitized adolescents, 10 of 26 were co-sensitized to nickel (Figure 5). Contact allergy reactions to the tested metals (nickel, cobalt and chromium) were more common among girls (11.6% versus 6.3% p<0.001). The difference was mainly driven by the numerically dominating contact allergy to nickel. Other substance groups did not differ significantly between the genders (Table 7).

Two percent (46/2285) of the patch tested adolescents were sensitized to more than one allergen; 0.1% (3/2285) were sensitized to three substances and 0.1% (3/2285) were sensitized to four substances.

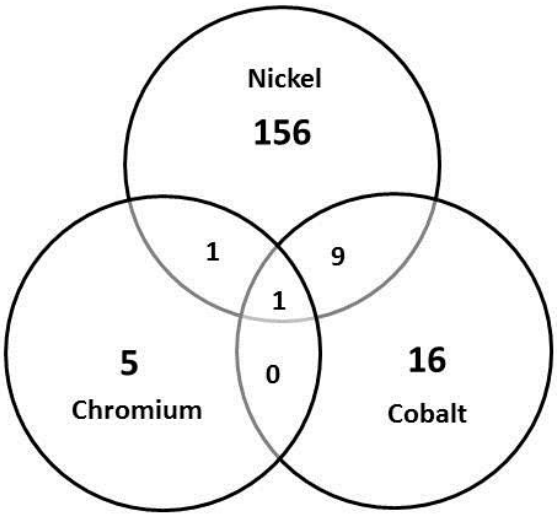

Figure 5. Venn diagram with detailed distribution of number of positive patch test reactions to the metals nickel, cobalt and chromium (n=200), showing solitary and concomitant reactions in patch tested adolescents with contact allergy to metals (n=188) (Paper I).

Table 7. Patch test substances divided into various substance groups, and prevalence of positive reactions day 2 among patch tested adolescents (n=2,285) in the BAMSE cohort at age 16 years (Study I).

	Prevalence			
Group	**All (%)**	**Girls (%)**	**Boys (%)**	***p***
Metals[a]	8.9	11.6	6.3	<0.001
Adhesives[b]	2.6	2.7	2.4	ns
Fragrances[c]	2.4	1.9	3.1	ns
Topical drugs[d]	1.2	1.6	0.8	ns
Hair dye[e]	1.1	1.1	1.0	ns
Preservatives[f]	1.1	0.8	1.4	ns
Rubber chemicals[g]	0.8	0.5	1.1	ns

[a] nickel sulfate, cobalt chloride, potassium dichromate. [b] *p*-tert butylphenol formaldehyde resin, colophonium, epoxy resin. [c] fragrance mix I, *Myroxylon pereirae.* [d] lanolin alcohol, tixocortol-21-pivalate, ethylenediamine 2HCl, caine mix, budesonide, hydrocortisone-17-butyrate, quinoline mix, neomycin sulfate. [e] *p*-phenylenediamine. [f] methylchloroisothiazolinone /methylisothiazolinone, formaldehyde, methyldibromo glutaronitrile, thiomersal, diazolidinyl urea, paraben mix, imidazolidinyl urea, quaternium-15. [g] carba mix, mercapto mix, mercaptobenzothiazole, thiuram mix, black rubber mix. ns, not significant. *p* for differences between girls and boys.

4.2 PREVALENCE OF SKIN EXPOSURES AND SKIN SYMPTOMS RELATED TO CERTAIN EXPOSURES (II)

The study population (n=3,115) was considered comparable with the original cohort for relevant background variables. Among the adolescents who completed the questionnaire at age 16 years, piercing was the most frequently reported skin exposure and significantly more girls than boys reported piercing. Hair dyeing was reported by 50.1% of the adolescents, with a significant female predominance. The prevalence of tattooing was 2.4% among the adolescents. No statistically significant difference was shown for tattooing, but somewhat more girls than boys reported having a tattoo. The prevalence rates of self-reported skin exposures are presented in Table 8.

Table 8. Prevalence of self-reported skin exposures among all responding adolescents (n=3,115) based on questionnaire at the 16-year follow-up in the BAMSE cohort (Adapted from paper II).

Skin exposure	Prevalence (%)			
	All	**Girls**	**Boys**	***p***
Piercing	55.4	92.7	16.5	<0.001
Hair dyeing	50.1	77.4	21.8	<0.001
Tattooing	2.4	2.9	1.8	0.061

p for differences between girls and boys.

The skin exposures to piercing, hair dyeing and tattooing were largely concomitant among girls, while solitary skin exposure to piercing and hair dyeing was more common among boys than concomitant skin exposure (Figure 6).

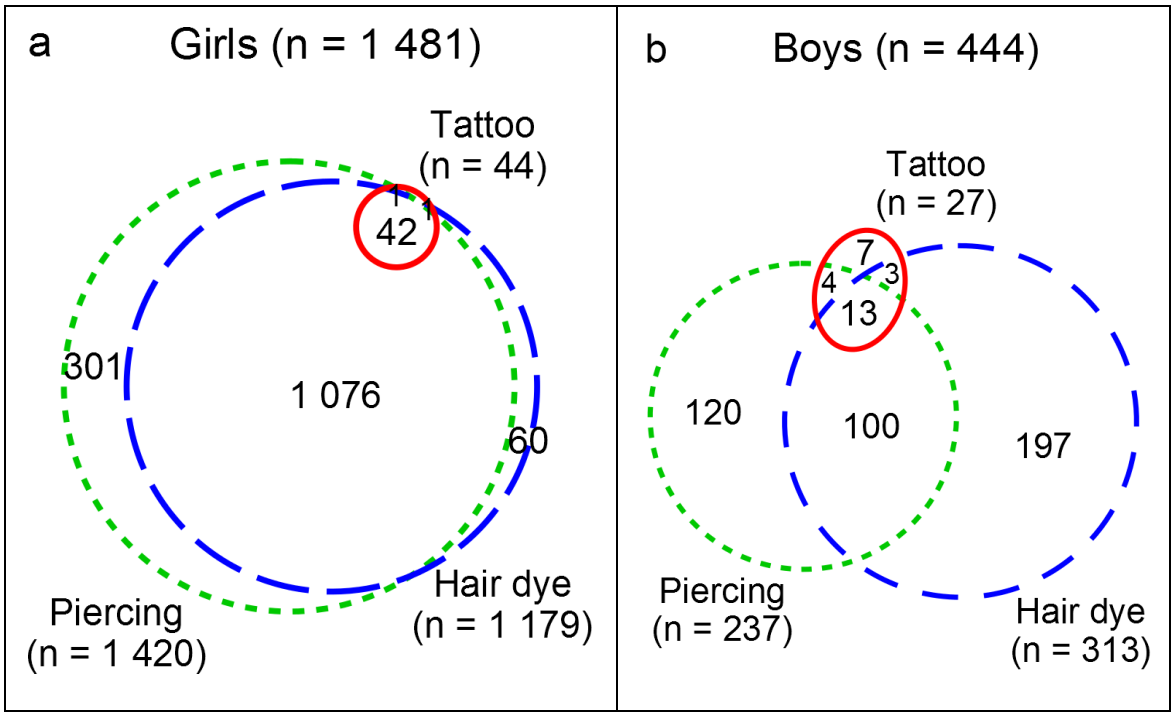

Figure 6. Euler diagrams presenting solitary and concomitant exposures of piercing, hair dyeing and tattooing among the adolescents completing the questionnaire regarding all these skin exposures (n=2,990). (a) among girls (n=1,481) (b) among boys (n=444) (Paper II).

More girls than boys reported skin symptoms, itchy rash or eczema related to contact with or use of specified items and consumer products. Skin symptoms after contact with undefined metal items was the most common self-reported skin symptom related to skin exposure, and piercing jewellery was the most commonly reported specified metal item to cause itchy rash. Self-reported itchy rash or eczema after use of makeup or personal hygiene products was the second most common skin symptom reported related to skin exposure and makeup or

perfume were the types of cosmetic product most frequently reported to cause this symptom. The prevalence of self-reported skin symptoms related to certain skin exposure are presented in Table 9.

Table 9. Selected self-reported skin symptoms from specified items or consumer products related to certain skin exposures among all adolescents responding (n=3,115) based on questionnaires answered by the adolescents at the 16-year follow-up in the BAMSE cohort (Adapted from paper II).

Skin symptoms	**Prevalence (%)**			
	All	**Girls**	**Boys**	***p***
Itchy rash from metal items	16.4	26.2	6.2	<0.001
Piercing jewellery	10.5	19.4	1.3	<0.001
Jewellery	6.6	10.3	2.8	<0.001
Metal in clothes	3.1	4.9	1.2	<0.001
Symptoms from hair dyeing	2.4	4.4	0.4	<0.001
Itchy rash from personal hygiene products or makeup	14.9	22.9	6.7	<0.001
Makeup or perfume	7.9	14.5	1.1	<0.001
Soap or shower gel	3.5	5.0	1.8	<0.001
Deodorant	2.6	3.4	1.7	0.003
Shampoo or conditioner	1.9	2.6	1.3	0.004

p for differences between girls and boys. Questions and response alternatives are specified in detail in supporting information, Table S1, to paper II.

4.3 ASSOCIATIONS BETWEEN SKIN EXPOSURES, SPECIFIC SKIN SYMPTOMS AND CONTACT ALLERGY AT AGE 16 YEARS (II)

The 2,285 adolescents that constituted the subpopulation for analyses were considered comparable with the original cohort despite small significant differences, with lower participation among boys and higher participation among adolescents whose parents were white-collar workers. The distribution of prevalence of specified skin exposures and skin symptoms was similar in the subpopulation and study population.

4.3.1 Nickel

Piercing was more common among adolescents who patch tested positive for nickel than among those who patch tested negative (73.5% versus 55.3%, p<0.001). Hair dyeing was also more commonly reported among adolescents with nickel positive reactions compared to nickel negative (64.5% versus 47.8%, p=0.022). The same was true for those who reported tattooing (4.2% versus 1.7%, p<0.001).

Itchy rash or eczema from contact with metal items was significantly more often reported by nickel positive than nickel negative adolescents (33.3% versus 15.7%, p<0.001). Also for some specified metal items, itchy rash was more commonly reported by nickel positive adolescents compared to nickel negative (jewellery for pierced holes: 21.8% versus 10.2%, p<0.001, other jewellery: 15.8% versus 6.1%, p<0.001, metal in clothes: 10.9% versus 2.7%, p<0.001).

Self-reported piercing was associated with an increased OR for nickel allergy (adjusted OR 1.77, 95% CI 1.04-3.03). When stratified by sex, self-reported piercing was associated with nickel allergy among boys but not statistically significant among girls (OR 1.99, 95% CI 1.05-3.75 and OR 1.43, 95% CI 0.61-3.38, respectively, p for interaction with sex = 0.55). Self-reported tattooing was also associated with increased OR for nickel allergy (adjusted OR 2.34, 95% CI 1.02-5.39).

Itchy rash from metal items was associated with nickel allergy (adjusted OR 2.25, 95% CI 1.57-3.23). Itchy rash in contact with specified metal items like metal in clothes, jewellery for pierced holes and other jewellery was also associated with an increased OR for nickel allergy.

4.3.2 Fragrance

Itchy rash from personal hygiene products or makeup was reported by 23.9% of fragrance positive adolescents compared to 15.3% of the fragrance negative. Reporting itchy rash from shampoo or conditioner was also more common among fragrance positive adolescents than fragrance negative (6.5% versus 2.0%, p=0.037) and similarly for reporting itchy rash from soap or shower gel (15.2% versus 3.6%, p<0.001). Itchy rash from personal hygiene products or makeup was associated with an increased OR for FM I allergy (adjusted OR 2.11, 95% CI 1.02-4.35). Itchy rash from soap and shower gel, and shampoo or conditioner was associated with increased OR for fragrance allergy, but no associations were found between itchy rash from deodorant, perfume or makeup.

4.3.3 PPD

Symptoms after hair dyeing were more common among adolescents with contact allergy to PPD than among those without (27.3% vs 4.8%, p = 0.001). Skin symptoms after hair dyeing were associated with an increased OR of contact allergy to PPD (adjusted OR 6.73, 95 % CI 1.82-24.96).

4.4 AD AT PRESCHOOL AGE AND CONTACT ALLERGY AT AGE 16 YEARS (III)

Adolescents with data on AD at preschool age and patch tested at age 16 years (n = 2,215) were included and the association between AD at preschool age and subsequent contact allergy in adolescence was studied. The study population was comparable with the original cohort in terms of relevant background variables, although small significant differences were observed for socioeconomic status and sex.

In a logistic regression analysis, AD at preschool age was not associated with an increased OR for contact allergy to any of the 30 patch tested substances (*any contact allergy)* (adjusted OR 1.04, 95% CI 0.81-1.35). However, when the analyses was stratified by sex, AD at preschool age was associated with *any contact allergy* in boys but not in girls (adjusted OR 1.51, 95% CI 1.03-2.02 and 0.77, 95% CI 0.54-1.10, respectively; p for interaction with sex = 0.012). AD at preschool age was not associated with contact allergy to nickel in either boys or girls. Further analysis showed that AD at preschool age was associated with an increased OR for contact allergy to FM I substances (adjusted OR 3.10, 95% CI 1.66-5.80). When girls and boys were analyzed separately AD at preschool age was associated with increased OR for contact allergy to FM I among both girls and boys, however not statistically significant among girls (adjusted OR 2.45, 95% CI 0.98-6.56 and adjusted OR 3.66, 95% CI 1.58-8.46, respectively).

An association was also present among individuals with AD in preschool age and IgE sensitization at 4 years with an increased OR for contact allergy to FM I (adjusted OR 3.80, 95% CI 1.67-8.61). In contrast, no association was seen among individuals with AD in preschool age without IgE sensitization at 4 years and contact allergy to FM I (adjusted OR 1.19, 95% CI 0.45-3.17).

In analysis of persistency of AD in childhood, children with AD at preschool age who also had AD at 8, 12 and/or 16 years (persistent AD) had an increased OR for contact allergy to FM I at 16 years of age (adjusted OR 3.82, 95% CI 1.77-8.22). For children with AD at preschool age and not thereafter (transient AD) OR was also increased for contact allergy to FM I, but this was not statistically significant (adjusted OR 2.29, 95% CI 0.98-5.36). No associations were found between persistent AD, transient AD or school onset AD and *any contact allergy* or to nickel allergy (Figure 7).

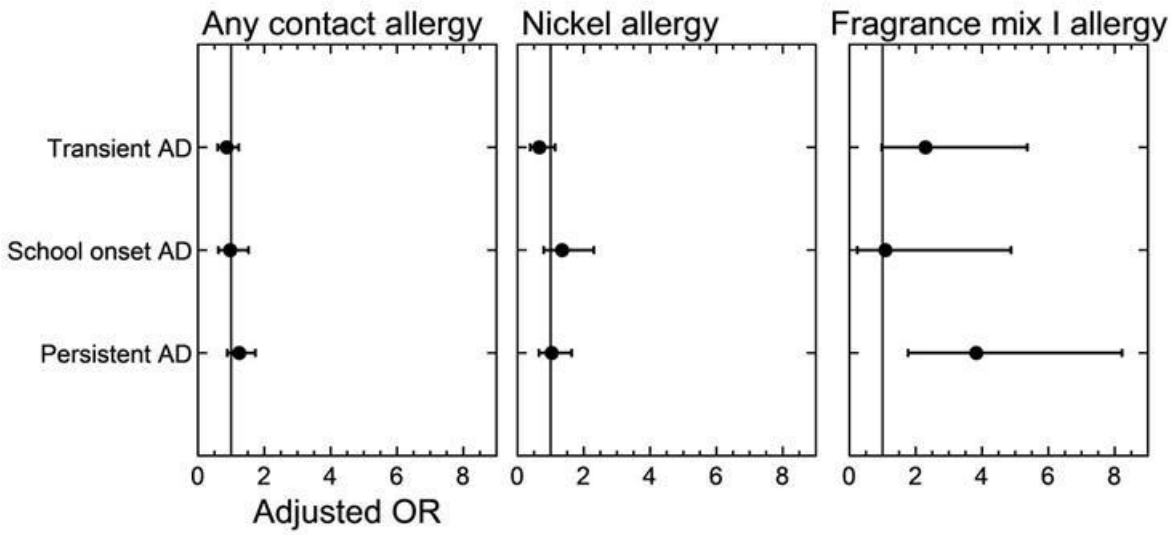

Figure 7. A logistic regression analysis of the association between the persistency of AD in childhood (transient AD, school onset AD and persistent AD) and contact allergy (*any contact allergy (n = 317)*, nickel allergy (n = 157) or fragrance mix I allergy (n = 41)) based on patch test at age 16 years. Adjustment was made for sex, parental atopic disease, parental smoking, breastfeeding, parental migration and socioeconomic status. AD, atopic dermatitis; OR, Odds ratio. (Paper III).

4.5 *FLG* MUTATIONS AND CONTACT ALLERGY, HAND ECZEMA AND DRY SKIN AT AGE 16 YEARS (IV)

Adolescents with data on patch test, hand eczema and *FLG* mutation status (1,822) were included in the study of *FLG* mutations and contact allergy, hand eczema or dry skin. The study population was compared to the original cohort and was considered comparable when comparing distribution of relevant background variables. Socioeconomic status and sex showed small statistically significant differences. The study population comprised 44.6% of the original cohort.

In adjusted logistic regression analysis, no significant associations were found between *FLG* mutations and *any contact allergy*, nickel allergy or FM I allergy at 16 years of age. In further adjusted logistic regression analyses, *FLG* mutations were not associated with hand eczema at 16 years of age or hand eczema ever. However, *FLG* mutations were significantly associated with dry skin at 16 years of age. Results are shown in detail in Table 10.

Table 10. Results from logistic regression analyses of association between filaggrin gene (*FLG*) mutations in blood tests and contact allergy in patch test at 16 years of age, *FLG* mutations and hand eczema according to questionnaire at 16 years, *FLG* mutations and dry skin according to questionnaire at 16 years. Adjusted for parental atopic disease, parental smoking and parental migration changing OR > 5%. (paper IV).

	***FLG* mutation**			
	No (n = 1695)[a]		Yes (n = 127)[a]	
	n	Adjusted OR (95% CI)	n	Adjusted OR (95% CI)
Contact allergy at 16 years[b]				
Any contact allergy	253	1.00 (ref.)	21	1.05 (0.62-1.76)
Nickel allergy	122	1.00 (ref.)	8	0.82 (0.37-1.80)
Fragrance allergy[c]	33	1.00 (ref.)	4	1.77 (0.61-5.14)
Hand eczema[d]				
Hand eczema at 16 years[e]	96	1.00 (ref.)	8	1.06 (0.48-2.34)
Hand eczema ever[f]	178	1.00 (ref.)	11	0.80 (0.41-1.56)
Dry skin[g] (N = 1814)[a]	No (n = 1687)[a]		Yes (n = 127)[a]	
Dry skin at 16 years	646	1.00 (ref.)	62	**1.50 (1.02-2.15)**

[a] N and n for dry skin at 16 years are lower due to missing values in the analysis. [b] According to patch test at 16 years. [c] Fragrance mix I. [d] According to questionnaire at 16 years. [e] An affirmative answer to the question, “Have you had hand eczema on any occasion during the past 12 months?”. [f] An affirmative answer to the question, “Have you ever had hand eczema (itching eruption, vesicles or itching rash)?” (79). [g] According to questionnaire at 16 years, an affirmative answer to the question, “Have you in the past 12 months had problems with dry skin?” Statistically significant result in bold. *FLG*: filaggrin gene; OR: odds ratio; CI: confidence interval; FM I, fragrance mix I.

5 DISCUSSION

5.1 MAIN FINDINGS AND INTERPRETATIONS

5.1.1 Prevalence of contact allergy among adolescents (I)

In study I, we describe the prevalence of contact allergy among adolescents in a population-based cohort. The prevalence of contact allergy to any of the 30 frequently occurring patch tested contact allergens was 15.3% among the patch tested adolescents. This is comparable with results from a study among teenagers and almost as high as in studies among adults, both studies performed in the general population in Denmark (1, 22). As previously reported among adults, adolescents and children, both among patients and in the general population, nickel was the most common cause of contact allergy (7.5%), particularly among females (9.8 versus 4.9, < 0.001) (17, 22, 30, 35). The higher prevalence of nickel allergy among girls reflects gender differences in skin exposures to nickel due to adornment behaviors, for example use of jewellery, piercing jewellery, clothes with applications or other items related to fashion rather than sex differences such as hormones (18, 46, 118). This higher skin exposure among girls is also shown in study II where girls more often report piercing and hair dyeing and more often report skin symptoms from contact with metal items and cosmetics, including personal hygiene products.

FM I, including fragrance substances, was after nickel the second most common cause of contact allergy, with a prevalence of 2.1%. These results are in line with previous studies among adults and adolescents in the general population, and pediatric patients patch tested at a dermatology clinic in Denmark (17, 54, 55). Fragrance allergy may be underestimated in study I, because it did not include FM II. TRUE test® did not include FM II at that time. FM II contains six more fragrance substances. It is likely that the prevalence of contact allergy to fragrance substances would have been higher if FM II had been used.

Prevalence of contact allergy to PTBP-FR was 1.9%. This is a higher prevalence than previously described in other population-based studies and may reflect an increasing skin exposure to PTBP-FR among children and adolescents (22). PTBP-FR is known to be a common cause of foot eczema and common sources of exposure are shoes and sport equipment for example shin guards and wet suits (119, 120).

Contact allergy to *Myroxylon pereirae* was more common among boys than girls. However, this result is based on only 8 adolescents who patch tested positive for *Myroxylon pereirae*. There was also an indication that contact allergy to FM I was more common among boys than girls; however, the difference was not statistically significant (2.4% versus 1.8%, p=0.29, Table 6). These differences are difficult to interpret, but might reflect gender differences concerning skin exposure to fragrance substances in certain personal hygiene products or chemical products (16).

Cobalt sensitization was not so often presented with co-sensitization to nickel as it has been previously described among adult patients with dermatitis (4, 48). These findings are based

on few positive patch test reactions among 2,285 patch tested adolescent. However, our finding is supported by similar findings by a recent study among adult patients (49).

Of the adolescents, 1.1% had contact allergy to PPD. This is consistent with studies among adults in the general population in Europe and likely reflects the frequent use of hair dye among adolescents as well as adults (61).

This study is strengthened by its large size with high participation rate: to the best of our knowledge this is the largest study published to date, in which a well-characterized cohort of adolescents in representative of the general population have been patch tested with a broad range of patch test substances. The main limitation is that patch test results were read only once, on day 2, instead of being read on day 2- 4 and on day 6-7, as is recommended in clinical practice. We assume that this will result in an underestimation of the true prevalence of contact allergy in this population-based sample. It is not considered to result in overestimation; thus, the results presented should be interpreted as minimum figures. This issue will be discussed in more detail under methodological considerations. Even interpreted with these limitations in mind, this study provides important new information about contact allergy prevalence and the distribution of contact allergy among adolescents in the general population.

5.1.1.1 Interpretations (I)

Contact allergy is common among adolescents and a patch test must always be contemplated when a child or adolescent has dermatitis of unknown cause or does not respond to suitable therapy, or obviously if contact allergy is suspected.

The high prevalence of nickel allergy among adolescents highlights the importance of trying to reduce skin exposures to nickel and complying with the EU nickel restriction to avoid future sensitization during childhood and adolescence.

Improved labelling of ingredients could be helpful in reducing risk for allergic contact dermatitis among adolescents who are aware of their contact allergy.

Reduced skin exposure to perfumed personal hygiene products during childhood may lower the risk of sensitization to fragrance substances.

5.1.2 Skin exposures and skin symptoms and the relation to contact allergy (II)

In study II, we report that piercing was common, particularly among girls (92.7%). These results were expected, and similar results have been reported previously in Sweden (46, 47, 105). Moreover, piercing was reported by a large proportion of the boys (16.5%) which is in line with previous reports in the general population (47, 105). There was an association between piercing and increased OR for nickel allergy, but stratified analysis showed that although piercing was associated with nickel allergy among boys, the link was not statistically significant among girls. These results are in line with results obtained among young adults in North America (121). The risk of nickel allergy related to piercing is

dependent on nickel release from jewellery used to pierce new holes or wear in already established pierced holes. If a non-sensitizing material is used the risk of contact allergy is low. In Sweden, the release of nickel from these products aimed for piercing is lower than in some other countries (40, 42). This may explain why an absolute majority of adolescent girls in this study reported piercing and yet only 9.8% had nickel allergy at age 16 years. The high levels of self-reported piercing among adolescent boys reflect an adornment behavior that can increase the risk of nickel allergy among adult men in the future. This development might be prevented if boys and men are advised to avoid skin sensitizing materials, and limit their exposure to skin sensitizing metals.

We found an association between reported itchy rash after skin contact with metal items and an increased OR for nickel allergy. This is in line with well-established knowledge that objects containing nickel can release nickel during skin contact and pose a risk of development of allergic contact dermatitis among individuals with contact allergy to nickel (11, 40).

Moreover, we report an association between itchy rash after use of personal hygiene products and increased OR for contact allergy to FM I. This association was found for rinse-off products like shampoo and soap but not for leave-on products like makeup, perfume and deodorants. An association between fragrance allergy and cosmetics was reported among adults in Denmark (122). As previously mentioned, our study probably underestimated fragrance allergy, because only FM I was tested, along with *Myroxylon pereirae* as fragrance markers and not FM II.

Use of hair dye was common among the adolescents (50.1%), more so among girls (77.4%) than boys (21.8%). These results resemble those from adults in the general population, but are somewhat higher than previously reported among adolescents in Sweden (61, 105, 107). Reporting adverse skin reactions after hair dye use was associated with contact allergy to PPD. Hair dyeing is since decades well established as a risk factor for contact allergy to PPD (61).

In this study we showed a low frequency of permanent tattoos and a previous survey showed similar results among 12-16-year-olds (105).

This study had a large sample size, and the questions were very detailed. However, multiple questions may be challenging for an adolescent to answer, moreover many questions about other allergy related diseases and general health were posed at the same time. This should be considered when interpreting the results. There were associations between reporting skin symptoms to various items and cosmetic products and contact allergy according to patch test in this study. This highlights that it is important to ask these questions in questionnaires as well as in the clinical situation, because they may give indications of contact allergy.

When we were performing this study we found very few previous studies done among adolescents with similar approach. Thus our study provides new, detailed knowledge about

skin exposures, skin symptoms and the associations with contact allergy among adolescents. However, more knowledge is needed and future studies will hopefully provide insights on how to tackle this problem for children and adolescents.

5.1.2.1 Interpretations (II)

Fashion trends with increased adornment behaviors like piercing, hair dyeing and tattooing can lead to higher risk of contact allergy and allergic contact dermatitis in the future.

Girls have much higher skin exposure than boys due to adornment behaviors like piercing, hair dyeing and tattooing with known risks for skin sensitization.

Adolescents do report skin problems as a consequence of adornments and consumer product use.

Improving the quality of consumer products by using non-sensitizing substances and materials, along with better labelling and information concerning allergy risks due to skin exposures, could prevent young children from developing skin sensitization early in life and later suffering consequences like allergic contact dermatitis.

5.1.3 AD at preschool age and contact allergy in adolescence (III)

In study III, we report indications of an association between AD at preschool age and contact allergy to FM I. This association was seen particularly among individuals with AD at preschool age in combination with IgE sensitization at 4 years, but not among individuals with AD at preschool age who lacked IgE sensitization. Contact allergy to FM I is prevalent among children and adolescents and our finding is in line with previous findings obtained among patch tested pediatric dermatology patients showing that patients with AD had higher prevalence of contact allergy to fragrance than patients without AD (52, 60). Individuals with AD may be exposed to topical tretments frequently and for longer treatment periods; moreover, moisturizers, soaps, wipes and other cosmetic and personal hygiene products intended for children are known to contain sensitizing fragrance substances (56, 57). This skin exposure in combination with inflamed skin with a deteriorated skin barrier among individuals with AD may explain or contribute to the association with fragrance allergy (66). The reported association between AD at preschool age in combination with early IgE sensitization and contact allergy to fragrances at 16 years may indicate that the impaired skin barrier common among individuals with AD facilitates penetration of “atopic” allergens (usually proteins) as well as contact allergens. However, the individuals with early IgE sensitization might also present with a more severe, long-lasting AD during childhood that could exacerbate long-term deterioration of the skin barrier (98).

In contrast, AD at preschool age was not associated with nickel allergy at 16 years of age. Previous studies describing the relation between AD and nickel allergy diverge; it has been suggested that since piercing is one cause of nickel allergy, piercing dominates as a source of nickel exposure and becomes more important than skin exposure on the defective skin barrier

(22, 36, 123). It is important to remember, however, that nickel exposure does not arise exclusively through piercing and piercing jewellery, but from many sources of nickel exposure (40). Nickel is released from toys and from other items with prolonged contact with the skin (38, 124). The EU restriction on nickel release has reduced the nickel exposure overall and can have reduced the skin exposure to nickel and thus the risk of nickel allergy among the adolescents in this study (34).

The reported association between AD at preschool age and *any contact allergy* among boys, which was not present among girls, might be influenced by the fact that contact allergy among girls is numerically dominated by nickel. Since there is no association between AD at preschool age and nickel one could speculate that this could "hide" associations to other specific contact allergens tested. This interpretation may be supported by a cross-sectional study describing an association between AD and contact allergy that emerged when nickel was excluded from the analysis based on the speculation that high prevalence of piercing might affect the results (125).

Moreover, AD at preschool age that persisted through childhood was significantly associated with contact allergy to fragrance. Contact allergy to fragrance can be misdiagnosed as AD, since it can cause persistent and relapsing dermatitis. Children with allergic contact dermatitis have been reported to meet the criteria for AD, and thus it can be difficult to distinguish these two diagnoses based on diagnostic criteria, without performing a patch test (32). Both epidemiological studies and clinical practice can be challenged by this. In a recently published study among dermatology patients a high frequency of contact allergy was found among both adults and children with recalcitrant AD (126). Persistent AD could also represent individuals with more severe disease, who have a defect skin barrier for long periods and undergo prolonged topical treatments.

The association between AD in preschool age and contact allergy to FM I is based on limited number of events and the results should be interpreted with that in mind. However, this study provides interesting information on an association between AD in early childhood and contact allergy to fragrances, but further studies are needed to examine this in detail. It would be preferable to included FM II in the patch test and to include individuals for whom data on AD in early childhood is available and patch test them in adulthood. If possible, a prospective cohort study design would be optimal.

5.1.3.1 Interpretation (III)

AD in early childhood may be associated with contact allergy to fragrance substances in adolescence.

AD in early childhood is not associated with nickel allergy in adolescence.

Avoidance of skin exposures to perfumed products containing sensitizing fragrance substances seems to be advisable for children with AD in early childhood.

The dysfunctional skin barrier in individuals with AD might represent a risk for contact allergy and thus these individuals should take extra precautions to avoid repeated and prolonged skin exposure to skin sensitizing substances.

5.1.4 *FLG* mutations and contact allergy, hand eczema or dry skin (IV)

In study IV we report an association between *FLG* mutations and self-reported dry skin at 16 years of age. Such associations have previously been reported at different ages (89, 95, 127, 128). However, we found no consistent associations between *FLG* mutations and hand eczema or contact allergy according to patch test. My interpretation is that our results do not rule out *FLG* mutations as risk factor for contact allergy or hand eczema: there are several reasons why adolescence is not the optimal time to study this association. Most adolescents are either not yet working or at the beginning of their occupational career. Thus occupational exposures to water and sensitizing substances are still minimal. Their skin exposure history is still short, reflecting only childhood and early adolescence, and even if adornment behaviors like piercing and hair dyeing are common among adolescents, the skin exposure resulting from these behaviors is brief compared to that in adults (1, 103, 129). Moreover, AD is known to be a prominent risk factor for hand eczema and this has been confirmed among adolescents (79, 130-132). It is likely that AD is the main risk factor for hand eczema in adolescence, and that contact allergy and wet work have a greater influence on the development of hand eczema later in life, since adults have higher occupational and household exposure to wet work, irritants and contact allergens.

Even though the study population was large (n=1,822) the small number of adolescents who had both contact allergy and *FLG* mutations, limits the possibilities for statistical analysis. The results should therefore be interpreted in context.

The fact that the study was performed in a population-based setting and not among patients with dermatological conditions can also have affected the results. It is possible that *FLG* mutations are more likely to be associated with hand eczema in patients with more severe disease. This notion is supported by a study that showed an association between *FLG* mutations and persistent problems with hand eczema that was self-reported among the general adult population (133).

FLG mutations are population-specific and numerous different mutations have been described in different parts of the world (88). In the BAMSE cohort only around 3% of the children had parents who were both born outside Europe; this should be kept in mind when analyzing for *FLG* mutations and when interpreting results (134). The results of this study provide new information about the relation between *FLG* mutations and contact allergy, hand eczema and dry skin that have not been clearly investigated. However, the associations between *FLG* mutations, contact allergy, hand eczema and dry skin need to be studied in far greater detail: their interactions appear to be exceedingly complex.

5.1.4.1 Interpretations (IV)

FLG mutations are associated with self-reported dry skin in adolescence.

FLG mutations are not clearly associated with contact allergy or hand eczema in adolescence.

5.2 METHODOLOGICAL CONSIDERATIONS

Results from studies must always be interpreted in the context of the methods used to obtain them. An attempt to discuss methodological considerations, strengths and weaknesses of all the studies in this thesis follows below.

5.2.1 Strengths and limitations

The strengths of the studies in this thesis are the relatively large study sizes and high participation rate over the years, and that they were performed in a well characterized cohort in a population-based longitudinal design. The population-based design gave us opportunities to study contact allergy among adolescents that are from the general population and with detailed prospectively collected data. The patch test was performed after the adolescent filled in the questionnaire but before the results of the questionnaire were available; they were thus unknown to the patch test readers. Most of the baseline data was collected already when the child was just 2 months old.

The limitations are that the patch test was read only once, after two days, a simplified patch test method, which is known to underestimate contact allergy. This simplified patch test procedure was chosen both to ensure a high participation rate and it was necessary to perform the study in our population-based setting. If the study had been performed with patch test reading on day 2 and on day 4 we might have had 19-30% more positive reactions to nickel, as previously described in a study were schoolgirls were tested with TRUE test® (135). Some patch test substances are more often positive already on day 2, for example FM I (136). Other patch test substances are reported to give positive reactions later. Nickel sulfate, tixocortol-21-pivalate, PTBP-FR and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) have been reported to give later positive patch test reactions (137). Nonetheless, our interpretation is that a test regime with two patch test readings would have reduced the participation rate substantially.

Definition of AD, hand eczema and dry skin was based only on questionnaire data. This will be discussed under misclassification. Another possible limitation is that adolescence might not be the optimal age to investigate contact allergy, since contact allergy can develop at any age and accumulates; the contact allergy prevalence is thus by nature higher among older populations and thus when studying associations to contact allergy, adulthood might be more suitable. Moreover, the adolescents were asked only if they had ever had a piercing, dyed hair or had a tattoo. No questions were asked regarding quantity, e.g. how many piercings they had or how often they dyed their hair. Such questions were not included due to lack of space in the questionnaire.

There are also considerations associated with observational studies that will be further discussed below.

5.2.2 Random errors

Random errors can occur regardless of study design and result in associations purely by chance. However, the risk of random errors decreases if the study size increases: a larger study size increases precision of the results. A study with small random errors has a high precision.

Given the relatively common outcomes studied in this thesis, the BAMSE cohort is a comparably large study. This theoretically reduces the risk of random errors. When stratified analyses lower the number of individuals in the analysis the results should be interpreted with caution, since these results are more likely to be affected by random errors. Wide confidence intervals can indicate that the statistical certainty is lower.

5.2.3 Systematic errors

These errors are independent of the size of the study. Systematic errors can be divided into selection bias, misclassification and confounding. Systematic errors reduce the internal validity of an observational study. It is important to try to reduce these types of errors, but they might be present and must be kept in mind when interpreting results. These errors can result in over- or underestimation of the associations found in the study.

5.2.3.1 Selection bias

Selection bias can occur during selection of participants for a study or when a factor affects the participation in the study. Selection bias can be present in different parts of the selection process. The studies in this thesis were at risk for selection bias at several time points. First, when the BAMSE cohort was established, 75% of the eligible infants were recruited. A study performed among non-participants and excluded groups showed that parental smoking was more common among the excluded families, but no other significant differences were found concerning parental atopic disease or other parameters known at that time to be important for atopic allergy development (114). Second, children were lost to follow-up over time. This could theoretically select children whose parents were more motivated to continue participating because they had atopic disease themselves, for instance or because the child had already developed an allergy. A third potentially risky time point was when the study populations were defined. However, in all studies we compared the study population with the original cohort concerning background characteristics. In all four studies, we found the study population comparable with the original cohort despite somewhat higher participation among girls and adolescents whose parents were white-collar workers, but in study II these small differences were only present in the subpopulation. Selection bias might thus be relatively unimportant in these studies examining associations.

The BAMSE cohort was not mainly initiated to study disease prevalence (114). When we determine the prevalence of contact allergy in study I and quantify skin exposure and skin

symptoms in study II it is important to understand that even if 2,285 were patch tested in study I and 3,115 adolescents completed the questionnaire in study II, this is 56% and 76% of the original cohort (n=4,089), but only 32% and 43% of the children born in the defined geographical recruitment region (n=7,221). Thus, our prevalence data may not reflect the true prevalence in this region. Given that white-collar worker parents were somewhat overrepresented among the patch tested adolescents, it is possible that the prevalence is an underestimation based on the speculation that non-participants and those actively excluded might have had different skin exposure during childhood due to socioeconomic and other factors.

5.2.3.2 Misclassification (Information bias)

If systematic errors are present in the measuring or the definition of exposure and/or outcome there is a risk of information bias or misclassification. For example, if a strict definition is used for contact allergy mild cases of the disease might be excluded and if a broad definition is used healthy individuals might be defined as having the disease (misclassification of outcome). In these studies, we have equated contact allergy with having a positive patch test, but due to the simplified patch test method in the research setting with patch test reading only day 2, we assume that this result underestimated the prevalence of contact allergy. If two patch test readings had been done, the prevalence would probably have been higher. The simplified patch test method introduces risk for non-differential misclassification. The results should be interpreted with this in mind.

The definition of AD can also be made strict or broad. Possible misclassification of exposure to AD cannot be excluded since our studies determine presence or absence of AD based on questionnaire data for diagnostic criteria (modified criteria from Williams et al), that were aimed for dermatologists in the clinical setting (66).

Misclassification can be divided in differential and non-differential misclassifications. Differential misclassification is when the misclassification differs between the study groups, and thus posing a risk of over- or underestimation of effects. Non-differential misclassification is random and presents a risk of hiding a true association. Differential misclassification of the exposure is reduced in our studies by the fact that the BAMSE cohort has a prospective design. This minimizes the risk of information bias since the information is collected before the development of the disease, for example AD in study III.

5.2.3.3 Confounding

Confounding is when the association between exposure and outcome is skewed by a third variable. The confounding factor should be related to both outcome and exposure, but not causally related to either one. Unless the results are adjusted for the confounding factor they will be inaccurate; either an under- or overestimation can occur. Confounding can be controlled by stratification or regression models. The regression model is better because it offers the possibility of adjustment for several confounding factors at once. In study II-IV we adjusted for potential confounders, but there is always a risk of over-adjustment and therefore

the crude analysis is important as a comparison. Even after attempts to control for confounding, a risk for residual confounding remains in observational studies. Overall in the studies included in this thesis, adjustment for potential confounders had limited influence on the results. Moreover, in study IV we chose not to adjust for AD and instead we performed stratified analysis for AD, to avoid over-adjustment.

5.2.4 Effect modification

Effect modification can also be called interaction. This interaction can be present when the association between the exposure and the outcome is dependent on a third factor. When this interaction is present the magnitude of the association will be inaccurate. To deal with interaction, a stratified analysis can be performed for the third factor and then be analyzed for the association, separately. This strategy has been used in study II-IV. For example, when we made logistic regression analysis for the association between AD at preschool age and *any contact allergy*, interaction with sex was analyzed $p = 0.012$ (study III). The analysis was then stratified by sex and the results were presented separately.

5.2.5 Generalizability

Generalizability describes the degree to which findings obtained within the selected study populations can be applied to other populations, its external validity. For the studies within this thesis, with their large study size, population-based design and limited selection bias, we consider the associations valid for populations in other northwestern Europe countries with similar standards of living. However, the prevalence rates of contact allergy, skin exposures and skin symptoms may not reflect the true prevalence in the predefined region since only 57% of the infants (4089 of 7221) were included and then even fewer in the studies I and II where prevalence proportions were presented. The generalizability of the prevalence should therefore be interpreted with that in mind. It is possible that the groups that did not respond or were actively excluded had a higher (or lower) skin exposure and thus a different risk for contact allergy due to differences in for example socioeconomic or other factors. However, our results of the prevalence of contact allergy are in line with those of a previous large study among the general population in Denmark (22). The results on skin exposure are in general agreement with those of a survey in Stockholm (105). One should also bear in mind that the population in the geographically predefined region is not the same today as when the cohort was initiated, for example because of moving and migration. In this context it is reasonable to believe that the contact allergy prevalence and skin exposures can be somewhat different today due to societal and other trends.

5.3 FUTURE PERSPECTIVES

This thesis was an attempt to explore contact allergy among adolescents at a population-based level. With these studies I have contributed only a small part of the total picture. Still, since the research area was not fully investigated I could nonetheless contribute with interesting new information that may help define the big picture of contact allergy among adolescents.

The knowledge gained from these studies can provide better possibilities for preventive measures aiming to reduce the risk contact allergy and its consequences among children, adolescents and adults. Before preventive measures can be designed, we need to know what children and adolescents who have developed these health conditions were exposed to: these studies give us a glimpse of what exposures we might fruitfully aim at reducing. Avoidance of harmful skin exposure to skin sensitizing substances can reduce the risk of future contact allergy, allergic contact dermatitis and hand eczema. The EU restriction on nickel release is one important attempt to reduce skin exposure to a sensitizing substance. These studies indicate that it would be a good idea to try to reduce skin exposure to fragrance substances among children and adolescents. Fragrance substances were the second most common cause of contact allergy among adolescents and AD at preschool age was associated with contact allergy with fragrance in adolescence. The skin exposure to fragrances that leads to contact allergy to fragrance substances already at an early age could be limited by restrictions in products intended for children, for example by legislation.

At the clinical level, children and adolescents with AD or recurrent dermatitis should more frequently be offered patch testing at a dermatology clinic or be referred to a dermatology clinic. An individual that is diagnosed with contact allergy may have better possibilities to avoid skin exposure to the contact allergen and thus reduce the risk of developing allergic contact dermatitis.

At school and in primary care, children and adolescents with contact allergy, hand eczema or AD should have an opportunity to receive medical guidance before choosing an educational program and a future occupation. The observation that AD at preschool age was associated with increased OR for sensitization to FM I could theoretically indicate that these adolescents might need information before choosing to train for an occupation involving frequent exposure to fragrance substances, for example hairdresser. This can reduce the risk of dermatitis, sick-leave and the need to change occupation later in life. It could also lead to less suffering for the individual patient and economical benefit for both the individual and society as a whole.

In the workplace, the high prevalence of contact allergy among adolescents results in a risk of chronic dermatitis and lower working capacity because of skin exposure to contact allergens. The work environment needs to adapt and occupational skin exposures to contact allergens must be reduced.

More knowledge about the complex interplay between *FLG* mutation, contact allergy, hand eczema, dry skin and AD is needed. More detailed knowledge gained from future studies could provide possibilities to reduce skin exposure to skin sensitizing substances to the individuals with the highest risk for contact allergy. This will allow us to provide better information to patients and parents regarding skin exposures among children and adolescents, and attempt to reduce early contact sensitization that can have lifelong consequences.

6 CONCLUSIONS

Among 16-year-old adolescents in Sweden, 15% are sensitized to commonly occurring contact allergens. Nickel allergy is the most common, affecting girls more often than boys. Fragrance mix I allergy is the second most common contact allergen followed by *p*-tert-butylphenol formaldehyde resin, cobalt and *p*-phenylenediamine. The findings imply that the prevalence of contact allergy in adolescents is at almost the same level as in adults, and with similar distribution between genders.

Piercing and hair dyeing are reported by the majority at 16 years, and by more girls than boys. More girls also report skin symptoms related to skin exposures. Piercing, and itchy rash from metal items, are associated with nickel allergy. Skin symptoms from hygiene products or makeup are associated with fragrance allergy and symptoms after hair dyeing with allergy to *p*-Phenylenediamine.

Atopic dermatitis at preschool age may be associated with contact allergy to fragrance at 16 years, particularly among those who had both atopic dermatitis at preschool age and IgE sensitization at 4 years. No association is observed between atopic dermatitis at preschool age and nickel allergy at 16 years.

Filaggrin gene mutations are associated with dry skin, but not with contact allergy or hand eczema at 16 years.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Bakgrund: Kontaktallergi är en typ av allergi som kan orsaka allergiskt kontakteksem om den som är allergisk kommer i hudkontakt med ämnet ifråga. Kontaktallergi är vanligt förekommande hos vuxna, men mindre kunskap finns om hur vanligt det är hos barn och ungdomar. Kontaktallergi orsakas av allergiframkallande ämnen som kommer i kontakt med huden. Överkänsligheten för ämnet som orsakar allergin är livslång. Det finns begränsad kunskap om ungdomars hudexponering för allergiframkallande ämnen, samt hur ofta de besväras av allergiskt kontakteksem. Det är inte heller klarlagt om atopiskt eksem (böjveckseksem) innebär ökad risk för kontaktallergi. Det är också oklart om mutation i genen för filaggrin, som ger torr hud och ökad förekomst av atopiskt eksem, även ger ökad risk för kontaktallergi eller handeksem.

Syfte: Avhandlingens syfte var att beskriva förekomsten av kontaktallergi hos ungdomar vid 16 års ålder, och hur vanligt det är med piercing, hårfärgning, tatuering och kliande hudutslag vid hudkontakt med vissa föremål och produkter. Vi ville också undersöka sambandet mellan olika hudexponeringar, atopiskt eksem i tidig barndom och kontaktallergi. Vi studerade även sambandet mellan mutationer i filaggringenen och kontaktallergi, handeksem respektive torr hud.

Metoder: Vi använde data från en svensk födelsekohort (BAMSE) med 4,089 deltagare som rekryterades ur befolkningen som spädbarn under perioden 1994-1996 inom ett definierat geografiskt område. Vid studiestarten besvarade föräldrarna frågor om bakgrundsfaktorer. Vid uppföljningar vid olika åldrar besvarade föräldrarna frågor om atopiskt eksem. Vid 16 års ålder fick ungdomarna frågor om piercing, hårfärgning och tatuering samt om kliande utslag vid kontakt med föremål och produkter. De genomgick även lapptest för att avgöra om de hade kontaktallergi (n = 2,225). Vid fyra års ålder togs blodprov som analyserades för IgE-antikroppar och vid 16 års ålder togs blodprov för analys av mutation i filaggringenen.

Resultat: Vid 16 års ålder uppvisade 15% av ungdomarna kontaktallergi mot något av de testade ämnena. Nickelallergi var vanligast och vanligare bland flickor. Näst vanligast var kontaktallergi mot parfymämnen. Piercing och hårfärgning rapporterades av majoriteten av ungdomarna och var vanligare bland flickor. Det var också vanligare att flickor rapporterade kliande utslag av kontakt med föremål och produkter. Piercing och kliande utslag av metallföremål var vanligare hos ungdomar med kontaktallergi mot nickel. Kliande utslag av smink och hygienprodukter var vanligare hos dem med kontaktallergi mot parfymämnen. Det fanns ett statistiskt samband mellan atopiskt eksem i tidig barndom och kontaktallergi mot parfymämnen, men inte mot nickel. Ett samband fanns också mellan mutationer i filaggringenen och torr hud vid 16 år, men inget samband med kontaktallergi eller handeksem.

Slutsats: Avhandlingen bidrar till ökad kunskap om kontaktallergi hos ungdomar och visar att kontaktallergi är vanligt förekommande hos ungdomar i Sverige. Många har också hudkontakt allergiframkallande ämnen. Ungdomar har besvär av kliande utslag vid kontakt

med vissa föremål och produkter och det är vanligare hos flickor. Atopiskt eksem i tidig barndom ökar eventuellt risken för kontaktallergi mot parfymämnen, men verkar inte öka risken för nickelallergi. Mutationer i filaggringenen ger ökad risk för torr hud, men vi ser ingen tydligt ökad risk för kontaktallergi eller handeksem vid 16 års ålder.

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